



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 48/00, 35/00, C12N 15/63</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/30742</b> <b>(43) International Publication Date:</b> 24 June 1999 (24.06.99)
<b>(21) International Application Number:</b> PCT/US98/25720 <b>(22) International Filing Date:</b> 11 December 1998 (11.12.98) <b>(30) Priority Data:</b> 60/069,579 12 December 1997 (12.12.97) US <b>(71)(72) Applicants and Inventors:</b> NALDINI, Luigi [IT/TT]; Corso Monte Cucco 144, I-10141 Torino (IT). SONG, Jin-Ping [CN/US]; 3455 Rambow Drive, Palo Alto, CA 84306 (US). <b>(74) Agents:</b> NAKAMURA, Dean, H. et al.; Sughrue, Mion, Zinn, Macpeak & Seas, PLLC, Suite 800, 2100 Pennsylvania Avenue, N.W., Washington, DC 20037-3213 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> THERAPEUTIC USE OF LENTIVIRAL VECTORS  <b>(57) Abstract</b>  A lentivirus vector inhibits propagation of a lentivirus in a cell.		

## THERAPEUTIC USE OF LENTIVIRAL VECTORS

### FIELD OF THE INVENTION

The invention relates to the use of lentiviral vectors in the treatment of a disease resulting from or associated with a lentivirus.

### BACKGROUND OF THE INVENTION

5           Retrovirus vectors are a common tool for gene delivery (Miller, Nature (1992) 357:455-460). The ability of retrovirus vectors to deliver an unrearranged, single copy gene into a broad range of rodent, primate and human somatic cells makes retroviral vectors well suited for  
10           transferring genes to a cell.

          Lentiviruses are complex retroviruses which, in addition to the common retroviral genes gag, pol and env, contain other genes with regulatory or structural function. The higher complexity enables the lentivirus to  
15           modulate the life cycle thereof, as in the course of latent infection.

          A typical lentivirus is the human immunodeficiency virus (HIV), the etiologic agent of AIDS. In vivo, HIV can infect macrophages, which are terminally  
20           differentiated cells that rarely divide. In vitro, HIV can infect primary cultures of monocyte-derived macrophages (MDM) as well as HeLa-Cd4 or T lymphoid cells arrested in the cell cycle by treatment with aphidicolin or  $\gamma$  irradiation.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

### SUMMARY OF THE INVENTION

The instant invention relates to the use of lentiviral vectors per se for a therapeutic benefit. The vector need not contain a transgene with antiviral activity.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts four graphs of Gag p24 antigen expression in human SupT1 lymphocytes transduced with lentiviral vector at different multiplicity of infection (M.O.I.; rectangles, triangles, ellipses) or in control non-transduced cells (lozenges) after infection with different amounts of HIV.

Figure 2 depicts Gag p24 antigen expression and cell survival after HIV infection of human primary CD4<sup>+</sup> lymphocytes transduced with either a lentiviral vector (triangles) or a murine leukemia virus based vector (squares) or non-transduced cells (diamonds).

### DETAILED DESCRIPTION OF THE INVENTION

The instant invention provides use of a lentiviral vector. The vector can be one which carries a foreign gene with an anti-viral activity, however, that is not a prerequisite in the practice of the instant invention. Thus, a vector per se can be used.

The lentiviral genome and the proviral DNA have the three genes found in retroviruses: gag, pol and env, which are flanked by two long terminal repeat (LTR) sequences. The gag gene encodes the internal structural (matrix, capsid and nucleocapsid) proteins; the pol gene encodes the RNA-directed DNA polymerase (reverse transcriptase), a protease and an integrase; and the env gene encodes viral envelope glycoproteins. The 5' and 3'

LTR's serve to promote transcription and polyadenylation of the virion RNA's. The LTR contains all other cis-acting sequences necessary for viral replication. Lentiviruses have additional genes including vif, vpr, tat, rev, vpu, nef and vpx (in HIV-1, HIV-2 and/or SIV).

Adjacent to the 5' LTR are sequences necessary for reverse transcription of the genome (the tRNA primer binding site) and for efficient encapsidation of viral RNA into particles (the Psi site). If the sequences necessary for encapsidation (or packaging of retroviral RNA into infectious virions) are missing from the viral genome, the cis defect prevents encapsidation of genomic RNA. However, the resulting mutant remains capable of directing the synthesis of all virion proteins.

The vectors of interest are those which have an intact 5' and 3' lentivirus LTR. A vector of interest contains a packaging signal sequence comprising the leader sequence downstream of the LTR and until the beginning of the gag gene. The vector may also contain an additional portion of the gag gene to enhance packaging. The vector of interest also includes a part of the env gene containing the Rev Response Element (RRE), and it may or may not include a splice acceptor site downstream of the RRE. The vectors of interest may contain one or more transgenes, or foreign nucleic acid, and preferably a transgene with anti-viral activity. However, a vector of interest need not contain a heterologous gene.

The heterologous or foreign nucleic acid sequence, the transgene, is linked operably to a regulatory nucleic acid sequence. As used herein, the term "heterologous" nucleic acid sequence refers to a sequence that originates from a foreign species, or, if from the same species, it may be substantially modified from the original form. Alternatively, an unchanged nucleic acid sequence that is

not expressed normally in a cell is a heterologous nucleic acid sequence.

5           The term "operably linked" refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. Preferably, the heterologous sequence is linked to a promoter, resulting in a chimeric gene. The heterologous nucleic acid sequence is preferably under control of either the viral LTR promoter-enhancer signals or of an internal promoter, and retained signals within the retroviral LTR can still bring about efficient expression of the transgene.

10           The foreign gene can be any transcribable nucleic acid of interest. Generally the foreign gene encodes a polypeptide. Preferably the polypeptide has some therapeutic benefit. The polypeptide may supplement deficient or nonexistent expression of an endogenous protein in a host cell. The polypeptide can confer new properties on the host cell, such as a chimeric signalling receptor, see U.S. Pat. No. 5,359,046. The artisan can determine the appropriateness of a foreign gene practicing techniques taught herein and known in the art. For example, the artisan would know whether a foreign gene is of a suitable size for encapsidation and whether the foreign gene product is expressed properly.

20           It may be desirable to modulate the expression of a gene regulating molecule in a cell by the introduction of a molecule by the method of the invention. The term "modulate" envisions the suppression of expression of a gene when it is over-expressed or augmentation of expression when it is under-expressed. Where a cell proliferative disorder is associated with the expression of a gene, nucleic acid sequences that interfere with the expression of a gene at the translational level can be

used. The approach can utilize, for example, antisense nucleic acid, ribozymes or triplex agents to block transcription or translation of a specific mRNA, either by masking that mRNA with an antisense nucleic acid or triplex agent, or by cleaving same with a ribozyme. The target of those molecules is the lentiviral RNA. Moreover, the RNA may be a sequence of the virus not present in the vector or that has been mutated in the vector.

Antisense nucleic acids are DNA or RNA molecules which are complementary to at least a portion of a specific mRNA molecule (Weintraub, Sci. Am. (1990) 262:40). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA forming a double-stranded molecule. The antisense nucleic acids interfere with the translation of the mRNA since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides or more are preferred since such are synthesized easily and are less likely to cause problems than larger molecules when introduced into the target cell. The use of antisense methods to inhibit the in vitro translation of genes is well known in the art (Marcus-Sakura, Anal. Biochem. (1988) 172:289).

The antisense nucleic acid can be used to block expression of a viral protein or a dominantly active gene product, such as amyloid precursor protein that accumulates in Alzheimer's disease. Such methods are also useful for the treatment of Huntington's disease, hereditary Parkinsonism and other diseases. Antisense nucleic acids are also useful for the inhibition of expression of proteins associated with toxicity.

Use of an oligonucleotide to stall transcription can be by the mechanism known as the triplex strategy since the oligomer winds around double-helical DNA, forming a

three-strand helix. Therefore, the triplex compounds can be designed to recognize a unique site on a chosen gene (Maher et al., Antisense Res and Dev. (1991) 1(3):227; Helene, Anticancer Drug Dis. (1991) 6(6):569).

5           Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode those RNA's, it is possible to engineer molecules that recognize  
10           and cleave specific nucleotide sequences in an RNA molecule (Cech, J. Amer. Med Assn. (1988) 260:3030). A major advantage of that approach is only mRNA's with particular sequences are inactivated.

15           It may be desirable to transfer a nucleic acid encoding a biological response modifier. Included in that category are immunopotentiating agents including nucleic acids encoding a number of the cytokines classified as "interleukins", for example, interleukins 1 through 12. Also included in that category, although not necessarily  
20           working according to the same mechanism, are interferons, and in particular gamma interferon ( $\gamma$ -IFN), tumor necrosis factor (TNF) and granulocyte-macrophage colony stimulating factor (GM-CSF). It may be desirable to deliver such nucleic acids to bone marrow cells or macrophages to treat  
25           inborn enzymatic deficiencies or immune defects. Nucleic acids encoding growth factors, toxic peptides, ligands, receptors or other physiologically important proteins also can be introduced into cells. The transgene also can be an inducible toxic molecule.

30           The method of the invention may also be useful for neuronal, glial, fibroblast or mesenchymal cell transplantation, or "grafting", which involves transplantation of cells infected with the recombinant lentivirus of the invention ex vivo, or infection in vivo



into the central nervous system or into the ventricular cavities or subdurally onto the surface of a host brain. Such methods for grafting will be known to those skilled in the art and are described in Neural Grafting in the Mammalian CNS, Bjorklund & Stenevi, eds. (1985).

For diseases due to deficiency of a protein product, gene transfer could introduce a normal gene into the affected tissues for replacement therapy, as well as to create animal models for the disease using antisense mutations. For example, it may be desirable to insert a Factor VIII or IX encoding nucleic acid into a lentivirus for infection of a muscle, spleen or liver cell.

The promoter sequence may be homologous or heterologous to the desired gene sequence. A wide range of promoters may be utilized, including a viral or a mammalian promoter, and an inducible promoter. Cell or tissue specific promoters can be utilized to target expression of gene sequences in specific cell populations. Suitable mammalian and viral promoters for the instant invention are available in the art.

Optionally during the cloning stage, the nucleic acid construct referred to as the transfer vector, having the packaging signal and the heterologous cloning site, also contains a selectable marker gene. Marker genes are utilized to assay for the presence of the vector, and thus, to confirm infection and integration. The presence of a marker gene ensures the selection and growth of only those host cells which express the inserts. Typical selection genes encode proteins that confer resistance to antibiotics and other toxic substances, e.g., histidinol, puromycin, hygromycin, neomycin, methotrexate etc. and cell surface markers.

The recombinant virus of the invention is capable of

transferring a nucleic acid sequence into a mammalian cell. The term, "nucleic acid sequence", refers to any nucleic acid molecule, preferably DNA, as discussed in detail herein. The nucleic acid molecule may be derived from a variety of sources, including DNA, cDNA, synthetic DNA, RNA or combinations thereof. Such nucleic acid sequences may comprise genomic DNA which may or may not include naturally occurring introns. Moreover, such genomic DNA may be obtained in association with promoter regions, poly A sequences or other associated sequences. Genomic DNA may be extracted and purified from suitable cells by means well known in the art. Alternatively, messenger RNA (mRNA) can be isolated from cells and used to produce cDNA by reverse transcription or other means.

Preferably, the recombinant lentivirus produced by the method of the invention is a derivative of human immunodeficiency virus (HIV).

The vectors of interests are produced using known methods. The vectors of interest can be introduced into cells either as the nucleic acid or encapsidated as a virus particle. An artisan is familiar with methods for encapsidating a lentiviral vector of interest. The vectors are introduced into target cells using methods known by those of skill in the art.

Thus, the vectors can be introduced into human cell lines by calcium phosphate transfection, lipofection or electroporation, generally together with a dominant selectable marker, such as neo, DHFR, Gln synthetase or ADA, followed by selection in the presence of the appropriate drug and isolation of clones. The selectable marker gene can be the transgene.

A likely means for transforming host cells with a vector of interest is by infecting cells with virus

particles carrying a vector of interest. Thus, the vector of interest would be encapsidated using known packaging systems, such as that taught in U.S. Pat. No. 5,686,279 and in Naldini et al. Science (1996) 272:263-267. Briefly, using either a stable packaging cell line or by transient transfection, the vector of interest is introduced into a cell which packages the vector of interest into viral particles. The virus particles are obtained from the culture medium, treated as known in the art to provide a virus preparation.

~~The target cell then is exposed to the virus preparation. That can be via in vivo administration means, wherein the virus preparation is administered to a host, for example, in a parenteral form. Alternatively, cells from the host can be retrieved and maintained in culture where those cells are exposed to the virus preparation. Once transformed, stably or not, the cells then can be returned to the host.~~

While the therapeutic benefit of the instant invention can be obtained by the vector per se, it is preferred that the vector carry a transgene. Preferably that transgene is one which itself has a therapeutic effect. Thus, the vectors of interest should have a place in current therapy of diseases associated with lentivirus.

Although the techniques used to construct vectors and the like are provided in standard resource materials which describe specific conditions and procedures, for convenience, the following paragraphs may serve as a guideline.

Construction of the vectors of the invention employs standard ligation and restriction techniques which are well understood in the art (see Maniatis et al., in Molecular Cloning: A Laboratory Manual, Cold Spring

Harbor Laboratory, N.Y., 1982). Isolated plasmids, DNA sequences or synthesized oligonucleotides are cleaved, tailored and religated in the form desired.

5 Site-specific DNA cleavage is performed by treating with the suitable restriction enzyme (or enzymes) under conditions which are understood in the art, and the particulars of which are specified by the manufacturer of the commercially available restriction enzymes, see, e.g. New England Biolabs, Product Catalog. In general, about 10 1  $\mu$ g of plasmid or DNA sequences is cleaved by one unit of enzyme in about 20  $\mu$ l of buffer solution. Typically, an excess of restriction enzyme is used to ensure complete digestion of the DNA substrate. Incubation times of about one hour to two hours at about 37°C are workable, although 15 variations can be tolerated. After each incubation, protein is removed by extraction with phenol/chloroform, which may be followed by ether extraction, and the nucleic acid recovered from aqueous fractions by precipitation with ethanol. If desired, size separation of the cleaved 20 fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques. A general description of size separations is found in Methods of Enzymology 65:499-560 (1980).

25 Restriction cleaved fragments may be blunt ended by treating with the large fragment of E. coli DNA polymerase I (Klenow) in the presence of the four deoxynucleotide triphosphates (dNTP's) using incubation times of about 15 to 25 minutes at 20°C in 50 mM Tris (pH 7.6) 50 mM NaCl, 6 mM MgCl<sub>2</sub>, 6 mM DTT and 5-10  $\mu$ M 30 dNTP's. The Klenow fragment fills in at 5' sticky ends but chews back protruding 3' single strands, even though the four dNTP's are present. If desired, selective repair can be performed by supplying only one of the dNTP's, or with selected dNTP's, within the limitations dictated by 35 the nature of the sticky ends. After treatment with

Klenow, the mixture is extracted with phenol/chloroform and ethanol precipitated. Treatment under appropriate conditions with S1 nuclease or Bal-31 results in hydrolysis of any single-stranded portion.

5            Ligations can be performed in 15-50  $\mu$ l volumes under the following standard conditions and temperatures: 20 mM Tris-Cl pH 7.5, 10 mM  $MgCl_2$ , 10 mM DTT, 33 mg/ml BSA, 10 mM-50 mM NaCl and either 40  $\mu$ M ATP, 0.01-0.02 (Weiss) units T4 DNA ligase at 0°C (for "sticky end" ligation) or  
10           1 mM ATP, 0.3-0.6 (Weiss) units T4 DNA ligase at 14°C (for "blunt end" ligation). Intermolecular "sticky end" ligations are usually performed at 33-100  $\mu$ g/ml total DNA concentrations (5-100 mM total end concentration). Intermolecular blunt end ligations (usually employing a  
15           10-30 fold molar excess of linkers) are performed at 1  $\mu$ M total ends concentration.

            Lentiviral vectors (Naldini et al., supra and Proc. Natl. Acad. Sci. (1996) 93:11382-11388) have been used to infect human cells growth-arrested in vitro and to  
20           transduce neurons after direct injection into the brain of adult rats. The vector was efficient at transferring marker genes in vivo into the neurons and long term expression in the absence of detectable pathology was achieved. Animals analyzed ten months after a single  
25           injection of the vector, the longest time tested so far, showed no decrease in the average level of transgene expression and no sign of tissue pathology or immune reaction. (Blomer et al., J. Virol. (1997) 71:6641-6649). An improved version of the lentiviral vector in which the  
30           HIV virulence genes env, vif, vpr, vpu and nef were deleted without compromising the ability of the vector to transduce non-dividing cells have been developed. The multiply attenuated version represents a substantial improvement in the biosafety of the vector (Zufferey  
35           et al. Nat. Biotech. (1997) 15:871-875).

Viral supernatants are harvested using standard techniques such as filtration of supernatants 48 hours post transfection. The viral titer is determined by infection of, for example,  $10^6$  NIH 3T3 cells or  $10^5$  HeLa cells with an appropriate amount of viral supernatant, in the presence of 8  $\mu$ g/ml polybrene (Sigma Chemical Co., St. Louis, MO). Forty-eight hours later, the transduction efficiency is assayed.

While not wanting to be bound to any posited hypothesis, it is believed the mechanism of the resistance was mapped to a post-integration step and shown to be dependent on an intact HIV LTR in the vector. On HIV infection of transduced cells, transcription from the vector LTR was enhanced greatly, resulting in increased expression of the transgene. Conceivably the vector RNA competes effectively with the viral RNA's both for binding the transactivators and for packaging by the budding viral particles, resulting in inhibition of viral replication and mobilization and spreading of the vector. Viral particles collected from the infected transduced cells were less infectious than virus collected from infected non-transduced cells, and transferred efficiently the transgene into naive cells.

Thus, expression of both the vector and the virus in the same cell is detrimental to viral replication, and result in mobilization and spreading of the transgene into selected target cells of HIV. That effect and the strong enhancement of transgene expression induced by HIV are significant advantages of an HIV-derived vector of anti-HIV gene therapy applications.

Thus, the instant vector will find use alone, either containing a transgene or not, and preferably the transgene has an antiviral activity; or in combination with another vector carrying a transgene with antiviral

activity, wherein the instant vector does or does not contain a transgene.

The viral particles can be further purified from the viral supernatants as known in the art.

5           The viral particles or vector nucleic acid can be administered to a host with a disorder associated with or caused by a lentivirus using known techniques.

10           Actual delivery of the vectors or particles is accomplished by using any physical method that will transport same into a host and into the target cell. As used herein, "vector", means both a bare recombinant lentiviral vector and recombinant lentiviral particle. Simply dissolving a vector in Hanks' balanced saline solution or phosphate buffered saline is sufficient to provide a solution useful for injection. There are no known restrictions on the carriers or other components that can be coadministered with the vector (although compositions that degrade the virion or polynucleotides thereof should be avoided in the normal manner with vectors).

15

20

25           Pharmaceutical compositions can be prepared as injectable formulations to be delivered intramuscularly, including implantable pumps (known by those of skill in the art and described, for example, in U.S. Pat. No. 5,474,552). Numerous formulations for injection are known and can be used in the practice of the instant invention. The vectors can be used with any pharmaceutically acceptable carrier for ease of administration and handling.

30           Such aqueous solutions can be buffered, if desired, and the liquid diluent first rendered isotonic with saline or glucose. Solutions of the vector as a free acid (DNA

contains acidic phosphate groups) or a pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. A dispersion of viral particles also can be prepared in glycerol, liquid polyethylene glycols and mixtures thereof and in oils. Under ordinary conditions of storage and use, the preparations contain a preservative to prevent the growth of microorganisms. The sterile aqueous media employed are obtainable by standard techniques well-known to those skilled in the art.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that administration by a syringe is possible. The formulation must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by use of agents delaying absorption, for example, aluminum monostearate and gelatin.



5 Sterile injectable solutions are prepared by incorporating the vector in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying which yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

15 The therapeutic compounds of this invention may be administered to a host alone or in combination with pharmaceutically acceptable carriers. As noted above, the relative proportions of active ingredient and carrier are determined by the solubility and chemical nature of the compound, chosen route of administration and standard pharmaceutical practice.

25 The dosage of the instant therapeutic agents which will be most suitable for prophylaxis or treatment will vary with the form of administration, the particular recombinant vector chosen and the physiological characteristics of the particular patient under treatment. Generally, small dosages will be used initially and, if necessary, will be increased by small increments until the optimum effect under the circumstances is reached. Exemplary dosages are within the range of  $10^8$  up to approximately  $5 \times 10^{15}$  particles in a total volume of 3-10 ml. (George, how does this sound to you?)

30 The invention now having been described in detail, provided hereinbelow are non-limiting examples

fold

include this ~~in~~ last sentence in (2)

1  
↓  
want  
at 8:28  
4/9/91

demonstrating various embodiments of the instant invention.

### Example 1

#### CONSTRUCTION OF THE LENTIVIRAL VECTORS

5       The lentiviral transfer vector plasmids were derived from the plasmid pHR'-CMV-LacZ described previously in Naldini et al., Science (1996) 272:263-267. Plasmid pHR'-CMV-Neo was derived by substituting the BamHI-XhoI fragment of pHR'-CMV-LacZ containing the E.coli LacZ gene  
10       with a BamHI-XhoI fragment containing the neomycin phosphotransferase gene of E.coli (Beck et al., Gene (1982) 19:327-336).

15       pHR2 is a lentiviral transfer vector in which 124 base pairs (bp) of nef sequences upstream of the 3' LTR in pHR have been replaced with a polylinker both to reduce HIV-1 sequences and to facilitate transgene cloning. pHR2 was derived from pHR'-CMV-LacZ by replacing the 4.6 kilobase (kb) ClaI-StuI fragment with an 828 bp ClaI-StuI fragment generated by PCR using pHR'-CMV-LacZ as the template and  
20       with the oligonucleotide primer, 5'-CCATCGATCACGAGACTAGTCCTACGTATCCCCGGGGACGGGATCCGCGGAATTCC GTTTAAGAC-3' (SEQ ID NO:\_\_\_\_\_) and the primer 5'-TTATAATGTCAAGGCCTCTC-3' (SEQ ID NO:\_\_\_\_\_) in a three part ligation with a 4.4 kb StuI-NcoI fragment and a 4.5 kb  
25       NcoI-ClaI fragment from pHR'-CMV-LacZ.

30       Plasmid pHR2-PGK-GFP was derived by cloning the XhoI-BamHI fragment of pRT43.3PGKF3 (WO 97/07225) containing the human PGK promoter (GenBank Accession number #M11958 nucleotides 2-516) and the BamHI-NotI fragment of plasmid of pEGFP1 (Clontech) containing a codon usage-optimized and improved version of the Green Fluorescent Protein (GFP) of A. victoria and a NotI-SacII linker, into the XhoI and SacII sites of pHR2.

Example 2

## INHIBITION OF HIV-1 REPLICATION OF LYMPHOCYTES TRANSDUCED BY THE LENTIVIRAL VECTOR

Human SupT1 T-lymphoblastoid cells were obtained by ATCC. Human CD4<sup>+</sup> primary blood lymphocytes (PBL) were separated from buffy coats from donations, stimulated with 2.5 µg/ml phytohemagglutinin or Dynal beads coated with OKT3 and CD28 antibodies for 2 days, then washed and cultured with 100 U/ml of interleukin 2 (Chiron) in AIM-V medium (Gibco). The SupT1 cells or PBL were transduced either with lentivector or a murine leukemia virus (MLV) vector carrying the same transgene overnight in the presence of 2 µg/ml polybrene, then washed and selected for transgene expression after 48 hrs.

All vectors were produced by transient transfection of 293T cells and pseudotyped with the VSV.G envelope as described previously (Naldini et al., Proc. Natl. Acad. Sci. (1996) 93:11382-88). Cells transduced with vectors carrying the neomycin resistance gene were selected in medium containing 1 mg/ml G418, then cultured in normal medium for virus challenge. Cells transduced with vectors carrying the green fluorescent protein (GFP) as transgene were selected by cell sorting.

The cells were challenged with increasing amounts of HIV virus. HIV-1 virions were produced either by 293T cells transfected with the proviral infectious molecular clone R8, or by SupT1 cells chronically infected with R8 virus. R8 is a lymphocytotropic HIV-1 hybrid of the HXB2-D and NL43 isolates that expresses all HIV reading frames (Gallay et al., Cell (1995) 83:569-576). The virus stock was titered on HeLa P4 cells and had an infectivity of 1,000 to 3,000 infectious units/ng p24. The cells were washed twice after overnight incubation with the virus in the presence of 2 µg/ml polybrene, and further cultured

for up to 3 weeks. Every 3-4 days, the conditioned medium was collected and HIV replication was determined by accumulation of HIV-1 Gag p24 in the medium by a commercially available ELISA kit (DuPont).

5 In the first experiment (see Figure 1), SupT1 cells transduced by lentiviral vector carrying the neomycin resistance gene, pHR'-CMV-Neo, were tested. HIV accumulated in control non-transduced cultures. On the other hand, in cells transduced by the lentiviral vector, 10 pHR2, HIV replication was detected only for the higher amounts of HIV and p24 accumulation was decreased dramatically and delayed. Similar results were obtained with three different SupT1 populations selected after transduction with the lentiviral vector at different 15 multiplicity of infection (M.O.I.). Moreover, no cytopathic effect was observed in lentivector transduced cells infected with up to 10 ng of HIV. In contrast, the non-transduced cultures developed cytopathic effect with all tested amounts of HIV.

20 The applicability of the inhibitory effect on HIV growth to primary cells and its specificity for lentiviral vectors were tested in another experiment (see Figure 2). CD4<sup>+</sup> PBL's were transduced with either lentivector (pHR2-PGK-GFP) or the MLV retrovector carrying the same 25 GFP transgene driven by the human PGK promoter, and sorted for transgene expression. The selected populations then were challenged with HIV virus as described above. Both the non-transduced cells (indicated in the figure by diamonds) and sorted cells transduced by the MLV retrovector (indicated by squares) yielded similar levels 30 of p24 antigen in the culture medium. However, the cells transduced by the lentiviral (indicated by triangles) yielded sharply reduced p24 even after inoculation with high doses of HIV (100 ng p24 equivalent of virus). 35 Moreover, there were twice as many cells transduced by the

lentivector surviving 13 days after infection than those transduced by the retrovector or non-transduced. In cells transduced by the lentivector, transgene expression was augmented significantly after infection with the HIV virus.

5

All publications and patent applications cited in this specification are herein incorporated by reference in their entirety as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

10

As will be apparent to those skilled in the art to which the invention pertains, the present invention may be embodied in forms other than those specifically disclosed above, for example to transfect and transduce other mammalian cell types, without departing from the spirit or essential characteristics of the invention. The particular embodiments of the invention described above, are, therefore, to be considered as illustrative and not restrictive. The scope of the present invention is as set forth in the appended claims rather than being limited to the examples contained in the foregoing description.

15

20

We claim:

1. A method for treating a host infected with a lentivirus comprising exposing said host to a lentivirus vector and a biologically acceptable carrier, excipient and diluent.

5

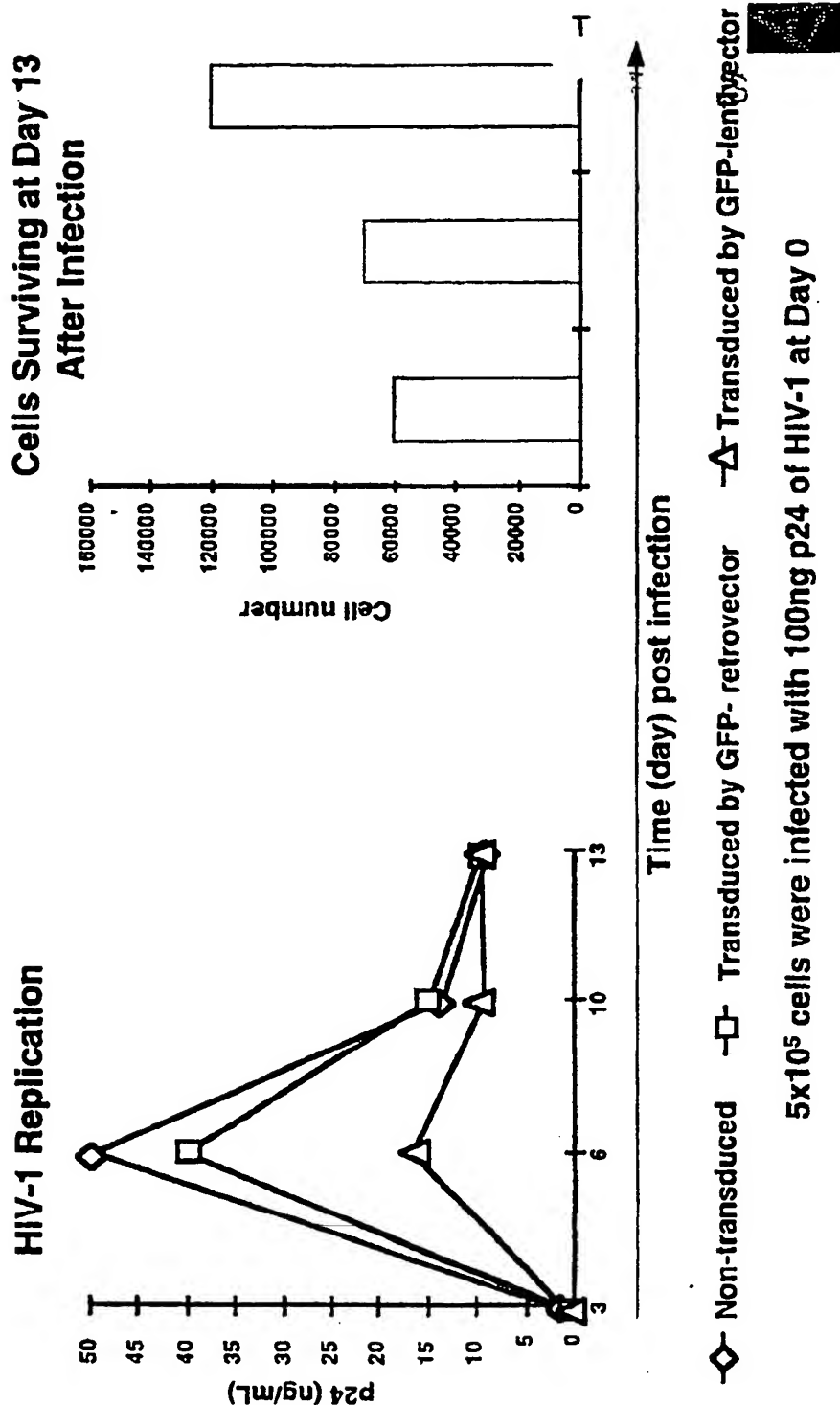
2. The method of claim 1, wherein said vector has an intact 5' LTR.

3. The method of claim 1, wherein said lentivirus is human immunodeficiency virus (HIV).

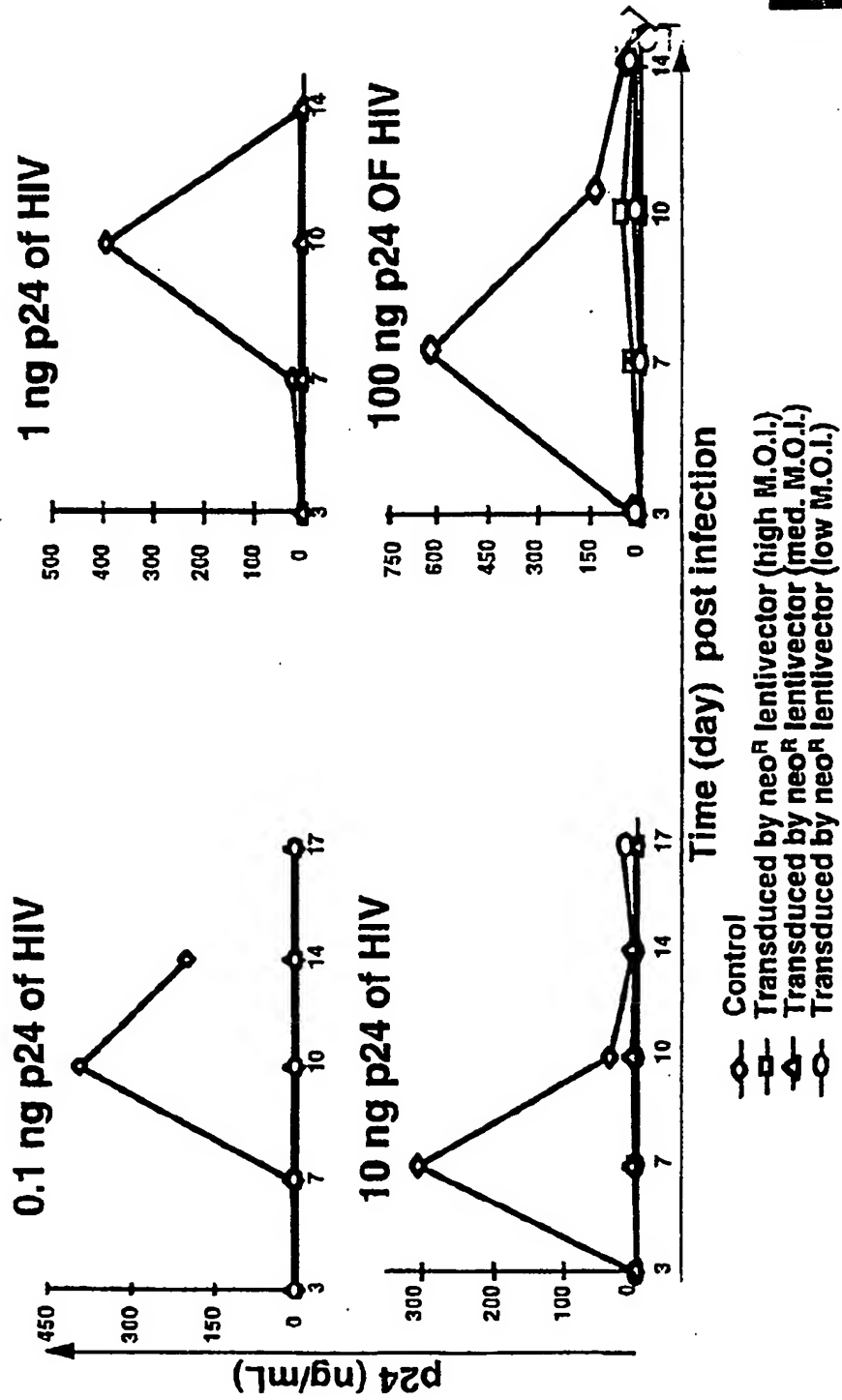
10

4. The method of claim 3, wherein said HIV is HIV-1.

# Inhibition of HIV Replication & Survival Advantage of Primary CD4<sup>+</sup> Lymphocytes Transduced by the Lentiviral Vector



# Inhibition of HIV Growth in SupT1 Cells Transduced by Lentiviral Vector





## SEQUENCE LISTING

<110> Song, Jin-Ping  
Naldini, Luigi  
Cell Genesys

<120> THERAPEUTIC USE OF LENTIVIRAL VECTORS

<130> F126422

<140>

<141>

<150> 60/069,579

<151> 1997-12-12

<160> 2

<170> PatentIn Ver. 2.0

<210> 1

<211> 65

<212> DNA

<213> primer

<400> 1

ccatcgatca cgagactagt cctacgtatc cccggggacg ggatccgcgg aattccgttt 60  
aagac 65

<210> 2

<211> 20

<212> DNA

<213> primer

<400> 2

ttataatgtc aaggcctctc

20

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/25720

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 48/00, 35/00; C12N 15/63

US CL : 514/44; 424/93.1; 435/320.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 424/93.1; 435/320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,650,309 A (WONG-STAAAL et al.) 22 July 1997, see entire document, especially column 2 and column 15, lines 54-60.	1-4
X	LISZIEWICZ et al. Inhibition of human immunodeficiency virus type 1 replication by regulated expression of a polymeric Tat activation response RNA decoy as a strategy for gene therapy in AIDS. PNAS USA, September 1993, Vol. 90, pages 8000-8004, see entire document.	1-4
A	BERKHOUT et al. Tat trans-activates the human immunodeficiency virus through a nascent RNA target. Cell 20 October 1989, Vol. 59, pages 273-282, entire document.	1-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents.	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

12 FEBRUARY 1999

Date of mailing of the international search report

25 FEB 1999

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

ANNE-MARIE BAKER, PH.D.

Telephone No. (703) 308-0196

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/25720

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS

DIALOG (file:medicine, USPatFull, Derwent, European Patents, JAPIO)

search terms: HIV, lentivir?, retrovir?, vector?, LTR, treat?, infect?